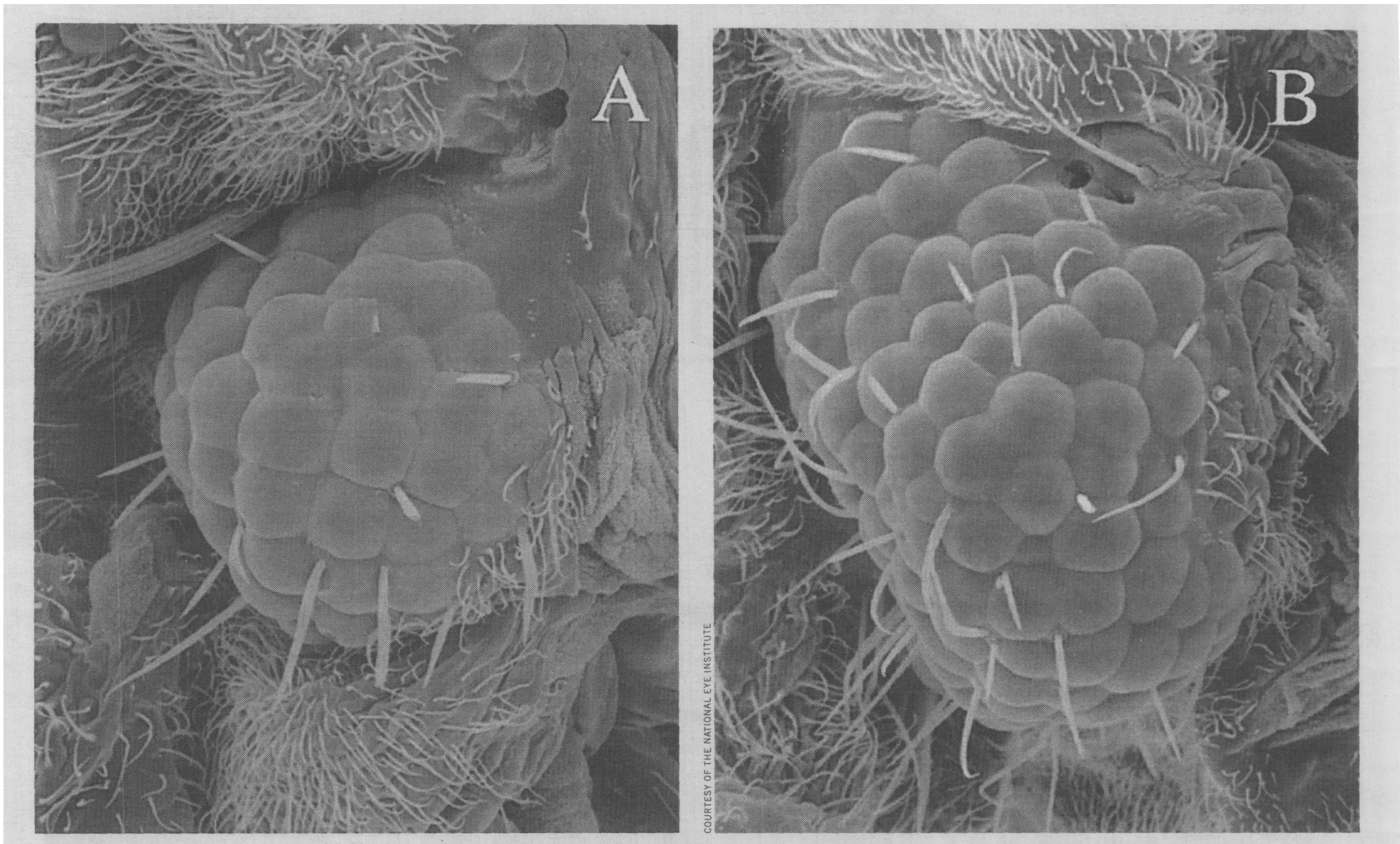
HOW GENETICS IS CHANGING OUR LIVES | PART TWO

THE WAY WE WERE



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Scientists produced these eyes on the wings of fruit flies. A is an eye generated by a squid's eye gene; B is one made with the fly's own gene.

WHAT DNA TELLS US ABOUT HOW LIFE EVOLVED

By Mark Schoofs

It was an experiment that could have come from a horror movie. Geneticist Walter Gehring took a gene that controls the development of eyes in mice and inserted it into fruit fly embryos, among cells that normally develop into legs. Legs they became—but with eyes all over them.

Researchers at the National Eye Institute recently repeated the experiment, this time splicing in the eye gene from a squid. The CONTINUED ON PAGE 38

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flies grew eyes on their wings, legs, and antennae—eyes that could actually respond to light. But because they were not wired to the brain, the flies could not see through them.

Such grotesque flies are more than insect versions of Frankenstein's monster. They are dramatic evidence that many genes are interchangeable among species, that vastly different organisms use similar genetic building blocks. In fact, the gene for eyes—called *Pax-6* in mice and squid—helps form the eyes of humans, too.

Researchers have found other genes common to various species. One is critical in the early development of limbs, from chicken wings to human arms to fruit fly legs (where it's called *sonic hedgehog*, after the cartoon character). Another gene, discovered just last month, regulates the body clocks of mice, humans, and flies. And a cluster of genes called *Hox* aligns the body axis of organisms as diverse as flies, worms, and mammals. Like a genetic compass, *Hox* genes tell the growing embryo where its head and tail are.

The *Hox* group usually consists *of* eight genes—and from flies to mice, they have even stayed in the same order, lined up on the long thread of DNA so the genes that act on head development are at one end and those that help build the posterior regions are at the other end. The individual *Hox* genes in mice and flies are not exactly the same—after all, the evolution of mammals and insects diverged more than 500 million years ago. But they are so similar—scientists say they are so "homologous"—that when researchers replaced two of the fly genes with the mouse counterparts, they worked just fine, and the flies developed normally.

The fact that very different organisms use basically the same genes has profound ramifications for our understanding of evolution. It suggests that eyes did not arise by the mutation of separate genes in different primordial creatures. Some scientists disagree with this interpretation, but many believe that all eyes in the animal world, from octopi to butterflies, probably arose from a single ancestor that may have used *Pax-6* to organize primitive light-receptor cells.

That is startling enough. But geneticists have discovered a second surprise: Organizing eyes is just one of many tasks that *Pax-6* carries out. It also helps construct the brain and olfactory organs, and squid tentacles. The same holds true for *Hox* genes, which not only orient the basic body axis but also contribute to the development of the jaw in certain animals and the brain in others. And *sonic hedgehog* doesn't just help make limbs, it also helps align the human central nervous system, telling cells where the top and bottom are.

Pax-6, *sonic hedgehog*, and the *Hox* cluster are all "regulatory genes." Instead of forming the tissues of the body, they generally act as switches, turning on and off the "structural genes," which do make tissue. "No one expected that organisms that looked very different—like flies and humans—would have same regulatory genes, and no one expected that making the nervous system and making limbs would use the same gene," says geneticist Rudolf Raff, of Indiana University. "These findings are real jaw droppers."

The old axiom "Ontogeny recapitulates phylogeny"—which holds that evolution is replayed in the development of every creature—is not literally true. But examining the regulatory switches in a developing organism illuminates how species diverge. Indeed, by observing the work of genes from fertilization to adulthood, scientists are forging new explanations for how life evolved, hypotheses that may well solve the most enduring riddles of evolution.

AROUND 560 MILLION years ago, there began a proliferation of life unmatched in history. Called the Cambrian explosion, it lasted 20 to 40 million years, a brief period from the standpoint of evolution. But during that interval, all 35 major body plans of contemporary animals emerged. There is some disagreement around the edges: Did all or almost all body plans arise during this period, and what exactly are the dates? Nevertheless, as recorded in the Burgess Shale, one of the world's richest fossil treasure troves, a mind-boggling diversity of life gushed forth during this era. For the first time, animals with skeletons, digestive cavities, and nervous systems arose. The brain of arthropods—a phylum that includes insects and crustaceans—may have been organized in as little as 5 million years.

How did all this happen? How did primitive, mostly microscopic organisms vault to large, complex animals? Even more intriguing, why have no new body plans emerged during the subsequent half billion years? The answer may well lie in the modular nature of DNA, and particularly its regulatory genes, the switches of life.

DNA is a chemical, and the development of a complex animal from a fertilized egg happens through a series of chemical interactions that are staggeringly intricate and exquisitely precise. Genes are sometimes nested within genes, and the same stretch of DNA often acts differently in different cells, or in the same cell at different stages of development. Why? Because each gene is activated and modulated by swarms of control molecules, proteins that attach to specific DNA segments like a lock and key. Some of these proteins are activated by signals which come from neighboring cells, which are getting input from still other cells, and so forth. "We haven't gotten anywhere near" understanding these complex regulatory webs, says leading geneticist Sydney Brenner. But this mystical process happens through the interaction of DNA with the body's other chemicals.

A gene, then, is simply a sequence of DNA that produces a chemical reaction—or a whole cascade of reactions. The powerful *Pax-6* "pulls enough levers to produce eye structures, even if it's put in a patch of cells that normally gives rise to legs," explains Cal Tech's Eric Davidson, a leading researcher of genetic regulatory systems. "If you take this same sequence, whether from a bird or a mouse, it will do the same thing. A sequence is a sequence is a sequence."

Indeed, the human brain has between 1 and 10 trillion neurons, whereas the worm *C. elegans* has exactly 302. But the genes in those 302 neurons make many of the same chemicals that are used in the human brain, including serotonin, the neurotransmitter targeted by Prozac. The implication for evolution is simple: Genes are modular. Like musical notes, they can produce incredible diversity depending on how they are orchestrated. "Everything has to do with when genes are turned on and off, and what genes they're associated with," says Davidson. "That's the whole secret of life."

He thinks that the Cambrian explosion was sparked by this kind of powerful switching. In a provocative hypothesis, he has suggested that the most important evolutionary step was the advent of "set-aside cells," cells that are not preordained to form one kind of tissue but can be recruited for almost any organ.

Primitive organisms—such as microscopic marine larvae, which probably existed just before the Cambrian explosion—develop by giving embryo cells strict and limited orders. Very soon after fertilization, cells are hard-wired to build the exterior membrane or some other structure. Utterly predetermined, these cells and their progeny cannot be redirected to do anything else. This kind of development is only found in small organisms of at most a few thousand cells.

But during the Cambrian explosion, large animals appeared. These embryos develop in a different way. Cells are set aside that have the potential to become part of any structure. As the embryo grows, these undifferentiated cells are recruited in stages. "It's as if you were designing a house," Davidson explains. "First you'd set out the order of all the rooms, then

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after everything was designed you'd put in the furniture and details."

Thus, the forelegs of a frog, the dorsal fin of some fish, and the wings of a chicken all begin with the same gene telling a group of cells that it will be a limb. "Only much later do batteries of genes that make bone and cartilage and skin come into play," says Davidson, "and then more that make, say, fingernails. Muscles are formed from cells that migrate in from the central part of body, but they obey the pattern set up by the limb bud."

How might set-aside cells have developed? This question cuts to the oldest conundrum in evolution. Natural selection can only refine existing traits; it cannot orginate new traits. That's one reason the late Japanese geneticist Motoo Kimura postulated that Darwin was wrong, that natural selection is not the driving force of evolution. Instead, he said, random mutations that naturally occur in DNA cause slight changes, most of which are "neutral"—they give the species neither an advantage nor a disadvantage. Over time, waves of these random, neutral mutations cause "genetic drift" that can create a new species. Thus, Kimura said, the law of nature is not survival of the fittest but "survival of the luckiest."

Kimura's "neutral theory of evolution" hasn't knocked Darwin off his pedestal—supposedly neutral changes might confer subtle advantages that natural selection would magnify—but random mutation and genetic drift are accepted as fact. Many external factors—such as viruses and industrial pollution—can also cause mutations. The recent outbreak of grotesquely deformed frogs—many missing eyes or legs—is now thought to be caused by some agent in the water.

Once set-aside cells developed, by whatever means, they could be molded in all kinds of novel ways. By reshuffling the DNA deck—turning existing genes on and off in subsequent stages and in novel arrangements—set-aside cells could be shaped into the precursors of almost any structure, be it brains or skeletons or eyes. Then natural selection could refine these innovations.

Hox genes provide an example of such gene reshuffling. In centipedes, two genes from the *Hox* group help construct every body segment except the head, but in the *Onycophora* worm the counterparts of those same genes are used only in the hindmost tip. The difference is primarily reorchestration, not new notes.

This discovery was noted in *Science*, which also reported something more dramatic. Japanese researchers took set-aside cells from newt and frog embryos and placed them in petri dishes. By merely adding a protein known to turn on organ formation, they induced the cells to form a heart—and even though it was in a petri dish, it was beating. To another group of set-aside cells, the researchers added a slightly higher dose of the protein, and the cells formed a liver. Eons of evolution were required to produce the genetic programs set in motion by this protein. Nevertheless, the experiment demonstrates how plastic set-aside cells are.

Whether Davidson is right—whether "the most important evolutionary novelty" was the rise of set-aside cells—is open to question. But the idea that extremely varied forms of life are created from the same genetic deck is hardly debated anymore. New genes certainly arose, but the Cambrian explosion happened primarily through reshuffling existing genes into new and more complex arrangements that created all the basic animal forms.

But once evolution had proceeded down a path, the genetic programs became ever more intricate, so radical change was stymied. Small refinements were easy—sharpening a beak or streamlining a fin. Changing a beak to a snout, or a fin to a leg, was difficult, though still possible, given enough time. But transfiguring an insect into a jellyfish—even though they once shared a common ancestor—became impossible. That's one reason why, in the half billion years since the Cambrian explosion, no new body plans have emerged.

In his book *The Shape of Life*, Raff quotes the novelist Italo Calvino:

When you're young, all evolution lies before you, every road is open to you, and at the same time you can enjoy the fact of being there on the rock, flat mollusk-pulp, damp and happy. If you compare yourself with the limitations that come afterwards, if you think of how having one form excludes other forms, of the monotonous routine where you finally feel trapped, well, I don't mind saying life was beautiful in those days.

Someday, says Raff, biologists might be able to retrace evolution's twists and turns by shuffling genes in an organism to see whether the change leads forward or backward to an evolutionary cousin. Of course, there are towering technical problems. Chief among them is the need for the right egg.

"DNA can't grow on its own," explains Raff. Indeed, the first molecular signals that set an embryo's DNA growing come from the mother, so researchers would need to synthesize the mother's egg, perhaps by commandeering one from a related organism. But in principle, some of the now extinct animals in the Burgess Shale could be reconstructed not, as in *Jurassic Park*, from DNA in amber but from reordering the genes in living animals.

In fact, a crude forerunner of this experiment is already common. David Galas used to direct the government's Human Genome Project, and he now runs a biotech company called Darwin Molecular. "We do work on the immune system," he says. When a human gene is found, "one of the first experiments is to knock it out in a mouse—make a mouse without the gene." By comparing the "knockout mouse" to a normal mouse, researchers can see what the gene does—in mice and humans. Even though the two mammals diverged from their common ancestor 100 million years ago, "every gene in the immune system that has been elucidated plays essentially the same role" in mice and humans.

"For example," he continues, "the X chromosome remains as a single chromosome in both mice and humans." Segments of the chromosome have been rearranged, "but this piece matches that one, or this one is inverted but matches that one." So how come humans and mice are so different? Because very small changes can have staggering results. Humans and chimpanzees share more than 98 per cent of their DNA, notes Raff. "How can they look so different? By using the same genes in slightly different ways."

A breathtaking example has been found by Yale brain researcher Pasko Rakic. The human cerebral cortex has 10 to 15 times as many cells as the macaque monkey's cerebral cortex. But this huge difference in brain size occurs mainly because of a tiny difference in the genetic regulatory program that directs the developing embryos. In monkeys, the brain's "founder cells" keep dividing until about 40 days after conception; in humans, those same cells divide until day 42 or 43. Because the total number of cells doubles with each division cycle, the extra two to three days give humans an exponentially larger brain.

Rakic hasn't identified the genetic switches which control this process, but he says it's possible just "one gene could make that difference." He quips: "It's a small step for the cell, but a giant leap for mankind." V

Part Three: Genetics and Race

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